

Serial No.: 10/718,359

Confirmation No.: 3660

Filed: November 20, 2003

For: NaCT AS A TARGET FOR LIFESPAN EXPANSION AND WEIGHT REDUCTION

Amendments to the Claims

This listing of claims replaces all prior versions, and listings, of claims in the above-identified application:

1-11. (Canceled)

12. (Currently amended) An isolated polypeptide, wherein the polypeptide is encoded by a polynucleotide that hybridizes to SEQ ID NO:5 under stringent hybridization conditions, wherein the polypeptide is capable of Na^+ -dependent transmembrane transport of citrate, wherein the polypeptide comprises amino acids 500 to 520 of the rat NaCT transporter polypeptide (SEQ ID NO:4), and

wherein stringent hybridization conditions are 6X SSC, 5X Denhardt, 0.5% sodium dodecyl sulfate (SDS), and 100 $\mu\text{g}/\text{ml}$ fragmented and denatured salmon sperm DNA hybridized overnight at 65°C and washed in 2X SSC, 0.1% SDS at least one time at room temperature for about 10 minutes followed by at least one wash at 65°C for about 15 minutes followed by at least one wash in 0.2X SSC, 0.1% SDS at room temperature for at least 3 to 5 minutes.

13. (Currently amended) The isolated polypeptide of claim 12, wherein the polypeptide comprises SEQ ID NO:6 wherein amino acid positions 496 to 516 of SEQ ID NO:6 have been replaced with amino acids 500 to 520 of the rat NaCT transporter polypeptide (SEQ ID NO:4).

14-19. (Canceled)

20. (Currently amended) An isolated polypeptide comprising an amino acid sequence having at least 95% identity to SEQ ID NO:6, wherein the polypeptide is capable of Na^+ -dependent transmembrane transport of citrate.

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and wherein in the polypeptide comprises amino acids 500 to 520 of the rat NaCT transporter polypeptide (SEQ ID NO:4).

21. (Currently amended) The isolated polypeptide of claim 20, wherein the encoded Na^+ -dependent transmembrane transport of citrate by the isolated polypeptide is modulated not stimulated by Li^+ .

22-26. (Canceled)

27. (Previously Presented) The isolated polypeptide of claim 20, wherein the polypeptide capable of Na^+ -dependent transmembrane transport of citrate requires multiple Na^+ ions for transport coupling.

28. (Previously Presented) The isolated polypeptide of claim 20, wherein the transmembrane transport of citrate is electrogenic.

29-49. (Canceled)

50. (Currently amended) The isolated polypeptide of claim 20, the polypeptide comprising an amino acid sequence having at least 99% identity to SEQ ID NO:6 wherein amino acid positions 496 to 516 of SEQ ID NO:6 have been replaced with amino acids 500 to 520 of the rat NaCT transporter polypeptide (SEQ ID NO:4).

51-56. (Canceled)

57. (Withdrawn) A method of identifying an agent that modifies transmembrane citrate transporter activity comprising:

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contacting a host cell expressing a transmembrane citrate transporter polypeptide of claim 20 with an agent;

measuring citrate transport into the host cell in the presence of agent;

and comparing citrate transport into the host cell in the presence of the agent to citrate transport into the host cell in the absence of the agent;

wherein a decreased transport of citrate into the host cell in the presence of the agent indicates the agent is an inhibitor of transmembrane citrate transporter activity;

wherein an increased transport of citrate into the host cell in the presence of the agent indicates the agent is a stimulator of transmembrane citrate transporter activity.

58-75. (Canceled)

76. (Withdrawn) A method of identifying an agent that modifies Na^+ -dependent transmembrane citrate transporter activity comprising:

contacting a host cell expressing a Na^+ -dependent transmembrane citrate transporter of claim 20 with an agent;

measuring the citrate-induced inward electrical current into the host cell in the presence of agent; and

comparing the citrate-induced inward electrical current into the host cell in the presence of the agent to the citrate-induced inward electrical current into the host cell in the absence of the agent;

wherein a decrease in the inward electrical current into the host cell in the presence of the agent indicates the agent is a blocker of Na^+ -dependent transmembrane citrate transporter activity;

wherein an increase in the inward electrical current into the host cell in the presence of the agent indicates the agent is a stimulator of Na^+ -dependent transmembrane citrate transporter activity.

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77. (Withdrawn) A method of identifying an agent that serves as a substrate of a Na^+ -dependent transmembrane citrate transporter comprising:

contacting a host cell expressing a Na^+ -dependent transmembrane citrate transporter of claim 20 with an agent; and

determining the entry of the agent into the cell via the Na^+ -dependent transmembrane citrate transporter in the presence of agent;

wherein entry of the agent via the Na^+ -dependent transmembrane citrate transporter indicates the agent is a substrate of a Na^+ -dependent transmembrane citrate transporter.

78. (Currently amended) A recombinant polypeptide, wherein the recombinant polypeptide is encoded by a polynucleotide that hybridizes to SEQ ID NO:5 under stringent hybridization conditions,

wherein the recombinant polypeptide is capable of Na^+ -dependent transmembrane transport of citrate,

wherein the polypeptide comprises amino acids 500 to 520 of the rat NaCT transporter polypeptide (SEQ ID NO:4), and

wherein stringent hybridization conditions are 6X SSC, 5X Denhardt, 0.5% sodium dodecyl sulfate (SDS), and 100 $\mu\text{g}/\text{ml}$ fragmented and denatured salmon sperm DNA hybridized overnight at 65°C and washed in 2X SSC, 0.1% SDS at least one time at room temperature for about 10 minutes followed by at least one wash at 65°C for about 15 minutes followed by at least one wash in 0.2X SSC, 0.1% SDS at room temperature for at least 3 to 5 minutes.

79. (Currently amended) The recombinant polypeptide of claim 78, wherein the recombinant polypeptide comprises SEQ ID NO:6 wherein amino acid positions 496 to 516 of SEQ ID NO:6 have been replaced with amino acids 500 to 520 of the rat NaCT transporter polypeptide (SEQ ID NO:4).

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80-81. (Canceled)

82. (Currently amended) A recombinant polypeptide comprising an amino acid sequence having at least 95% identity to SEQ ID NO:6,

wherein the polypeptide is capable of Na^+ -dependent transmembrane transport of citrate,

and wherein in the polypeptide comprises amino acids 500 to 520 of the rat NaCT transporter polypeptide (SEQ ID NO:4).

83. (Currently amended) The recombinant polypeptide of claim 82, wherein the Na^+ -dependent transmembrane transport of citrate is modulated by Li^+ polypeptide comprising SEQ ID NO:6 wherein amino acid positions 496 to 516 of SEQ ID NO:6 have been replaced with amino acids 500 to 520 of the rat NaCT transporter polypeptide (SEQ ID NO:4).

84. (New) An isolated polypeptide, wherein the polypeptide is encoded by a polynucleotide that hybridizes to SEQ ID NO:5 under stringent hybridization conditions,

wherein the polypeptide is capable of Na^+ -dependent transmembrane transport of citrate,

wherein the polypeptide comprises a leucine at amino acid position 500,

and wherein stringent hybridization conditions are 6X SSC, 5X Denhardt, 0.5% sodium dodecyl sulfate (SDS), and 100 $\mu\text{g}/\text{ml}$ fragmented and denatured salmon sperm DNA hybridized overnight at 65°C and washed in 2X SSC, 0.1% SDS at least one time at room temperature for about 10 minutes followed by at least one wash at 65°C for about 15 minutes followed by at least one wash in 0.2X SSC, 0.1% SDS at room temperature for at least 3 to 5 minutes.

85. (New) The isolated polypeptide of claim 84, wherein the polypeptide comprises SEQ ID NO:6 wherein amino acid at position 500 has been changed from a phenylalanine to a leucine.

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86. (New) An isolated polypeptide, the isolated polypeptide comprising an amino acid sequence having at least 95% identity to SEQ ID NO:6,

wherein the polypeptide is capable of Na^+ -dependent transmembrane transport of citrate, and wherein the polypeptide comprises a leucine at amino acid position 500.

87. (New) The isolated polypeptide of claim 86, the polypeptide comprising SEQ ID NO:6 wherein the amino acid at position 500 of SEQ ID NO:6 has been changed from a phenylalanine to a leucine.

88. (New) An isolated polypeptide, the polypeptide comprising SEQ ID NO:6 wherein amino acid positions 496 to 516 of SEQ ID NO:6 have been replaced with amino acids 500 to 520 of the rat NaCT transporter polypeptide (SEQ ID NO:4).

89. (New) The isolated polypeptide of claim 88, wherein the polypeptide is capable of Na^+ -dependent transmembrane transport of citrate.

90. (New) An isolated polypeptide, the polypeptide comprising SEQ ID NO:6 wherein the amino acid at position 500 of SEQ ID NO:6 has been changed from a phenylalanine to a leucine.

91. (New) The isolated polypeptide of claim 90, wherein the polypeptide is capable of Na^+ -dependent transmembrane transport of citrate.

92. (New) A recombinant polypeptide, wherein the polypeptide is encoded by a polynucleotide that hybridizes to SEQ ID NO:5 under stringent hybridization conditions,

wherein the polypeptide is capable of Na^+ -dependent transmembrane transport of citrate, and wherein the polypeptide comprises a leucine at amino acid position 500,

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and wherein stringent hybridization conditions are 6X SSC, 5X Denhardt, 0.5% sodium dodecyl sulfate (SDS), and 100 µg/ml fragmented and denatured salmon sperm DNA hybridized overnight at 65°C and washed in 2X SSC, 0.1% SDS at least one time at room temperature for about 10 minutes followed by at least one wash at 65°C for about 15 minutes followed by at least one wash in 0.2X SSC, 0.1% SDS at room temperature for at least 3 to 5 minutes.

93. (New) The recombinant polypeptide of claim 92, wherein the polypeptide comprises SEQ ID NO:6 wherein the amino acid at position 500 has been changed from a phenylalanine to a leucine.

94. (New) A recombinant polypeptide, the polypeptide comprising an amino acid sequence having at least 95% identity to SEQ ID NO:6,

wherein the polypeptide is capable of Na⁺-dependent transmembrane transport of citrate,
and wherein the polypeptide comprises a leucine at amino acid at position 500.

95. (New) The recombinant polypeptide of claim 94, the polypeptide comprising SEQ ID NO:6 wherein the amino acid at position 500 of SEQ ID NO:6 has been changed from a phenylalanine to a leucine.

96. (New) A recombinant polypeptide, the polypeptide comprising SEQ ID NO:6 wherein amino acid positions 496 to 516 of SEQ ID NO:6 have been replaced with amino acids 500 to 520 of the rat NaCT transporter polypeptide (SEQ ID NO:4).

97. (New) The isolated polypeptide of claim 96, wherein the polypeptide is capable of Na⁺-dependent transmembrane transport of citrate.

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98. (New) A recombinant polypeptide, the polypeptide comprising SEQ ID NO:6 wherein the amino acid at position 500 of SEQ ID NO:6 has been changed from a phenylalanine to a leucine.

99. (New) The isolated polypeptide of claim 98, wherein the polypeptide is capable of Na^+ -dependent transmembrane transport of citrate.

100. (New) The polypeptide of claim 12, wherein the Na^+ -dependent transmembrane transport of citrate by the isolated polypeptide is not stimulated by Li^+ .

101. (New) The polypeptide of claim 78, wherein the Na^+ -dependent transmembrane transport of citrate by the isolated polypeptide is not stimulated by Li^+ .

102. (New) The polypeptide of claim 82, wherein the Na^+ -dependent transmembrane transport of citrate by the isolated polypeptide is not stimulated by Li^+ .

103. (New) The polypeptide of claim 84, wherein the Na^+ -dependent transmembrane transport of citrate by the isolated polypeptide is not stimulated by Li^+ .

104. (New) The polypeptide of claim 86, wherein the Na^+ -dependent transmembrane transport of citrate by the isolated polypeptide is not stimulated by Li^+ .

105. (New) The polypeptide of claim 92, wherein the Na^+ -dependent transmembrane transport of citrate by the isolated polypeptide is not stimulated by Li^+ .

106. (New) The polypeptide of claim 94, wherein the Na^+ -dependent transmembrane transport of citrate by the isolated polypeptide is not stimulated by Li^+ .

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107. (New) The polypeptide of claim 12, wherein the transmembrane transport of citrate is electrogenic.

108. (New) The polypeptide of claim 78, wherein the transmembrane transport of citrate is electrogenic.

109. (New) The polypeptide of claim 82, wherein the transmembrane transport of citrate is electrogenic.

110. (New) The polypeptide of claim 84, wherein the transmembrane transport of citrate is electrogenic.

111. (New) The polypeptide of claim 86, wherein the transmembrane transport of citrate is electrogenic.

112. (New) The polypeptide of claim 92, wherein the transmembrane transport of citrate is electrogenic.

113. (New) The polypeptide of claim 94, wherein the transmembrane transport of citrate is electrogenic.

114. (New) The polypeptide of claim 27, wherein the stoichiometry of transport coupling is 4:1.

115. (New) The polypeptide of claim 12, wherein the polypeptide capable of Na⁺-dependent transmembrane transport of citrate requires multiple Na⁺ ions for transport coupling.

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116. (New) The polypeptide of claim 115, wherein the stoichiometry of transport coupling is 4:1.

117. (New) The polypeptide of claim 78, wherein the polypeptide capable of Na⁺-dependent transmembrane transport of citrate requires multiple Na⁺ ions for transport coupling.

118. (New) The polypeptide of claim 117, wherein the stoichiometry of transport coupling is 4:1.

119. (New) The polypeptide of claim 82, wherein the polypeptide capable of Na⁺-dependent transmembrane transport of citrate requires multiple Na⁺ ions for transport coupling.

120. (New) The polypeptide of claim 119, wherein the stoichiometry of transport coupling is 4:1.

121. (New) The polypeptide of claim 84, wherein the polypeptide capable of Na⁺-dependent transmembrane transport of citrate requires multiple Na⁺ ions for transport coupling.

122. (New) The polypeptide of claim 121, wherein the stoichiometry of transport coupling is 4:1.

123. (New) The polypeptide of claim 86, wherein the polypeptide capable of Na⁺-dependent transmembrane transport of citrate requires multiple Na⁺ ions for transport coupling.

124. (New) The polypeptide of claim 123, wherein the stoichiometry of transport coupling is 4:1.

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125. (New) The polypeptide of claim 92, wherein the polypeptide capable of Na⁺-dependent transmembrane transport of citrate requires multiple Na⁺ ions for transport coupling.
126. (New) The polypeptide of claim 125, wherein the stoichiometry of transport coupling is 4:1.
127. (New) The polypeptide of claim 94, wherein the polypeptide capable of Na⁺-dependent transmembrane transport of citrate requires multiple Na⁺ ions for transport coupling.
128. (New) The polypeptide of claim 127, wherein the stoichiometry of transport coupling is 4:1.
129. (New) The polypeptide of claim 12, wherein the polypeptide transports the tricarboxylate citrate with a higher affinity than a dicarboxylate.
130. (New) The polypeptide of claim 20, wherein the polypeptide transports the tricarboxylate citrate with a higher affinity than a dicarboxylate.
131. (New) The polypeptide of claim 78, wherein the polypeptide transports the tricarboxylate citrate with a higher affinity than a dicarboxylate.
132. (New) The polypeptide of claim 82, wherein the polypeptide transports the tricarboxylate citrate with a higher affinity than a dicarboxylate.
133. (New) The polypeptide of claim 84, wherein the polypeptide transports the tricarboxylate citrate with a higher affinity than a dicarboxylate.

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134. (New) The polypeptide of claim 86, wherein the polypeptide transports the tricarboxylate citrate with a higher affinity than a dicarboxylate.

135. (New) The polypeptide of claim 92, wherein the polypeptide transports the tricarboxylate citrate with a higher affinity than a dicarboxylate.

136. (New) The polypeptide of claim 94, wherein the polypeptide transports the tricarboxylate citrate with a higher affinity than a dicarboxylate.